SEQ ID NO: 19

Ser Cys Asp Lys Asn Thr Gly Asp Tyr Tyr Gly Asp Ser Tyr Glu Asp;

(h) the epitope leucine 730 to serine 741 inclusive, defined by the following sequence:

SEQ ID NO: 20:

Leu Leu Ser Lys Asn Asn Ala Ile Glu Pro Arg Ser;

(i) the epitope of (h) wherein the terminal amino acid serine and/or the first amino acid leucine are deleted;

(j) the epitope serine 817 to serine 830 inclusive, defined by the following sequence:

SEQ ID NO: 21:

Ser Asp Asp Pro Ser Gly Ala Ile Asp Ser Asn Asn Ser; and

(k) any of the preceding epitopes (a) to (j) wherein at least one amino acid has been deleted.

IN THE SEQUENCE LISTING:

Please add the accompanying Sequence Listing to the application.

REMARKS

The specification has been amended to change "Gly" to "Glu" on page 8 of the specification. Support for this amendment is found on page 8, line 26 where it states, "the epitope glutamic acid 1885 to alanine 1901 inclusive, defined by the following sequence:" and page 18, line 30 of the specification to page 19, line 3 of the specification where "Glu" appears at the beginning of the amino acid sequence. Claim 6 has been amended to include the same revision.

The typographical error in which the amino acid tyrosine was abbreviated as "Try" within SEQ ID NO: 19 on page 11, line 8 and page 21, line 12 has been amended to recite the abbreviation "Tyr". Claim 10 was amended to include the same revision.

The sequence of SEQ ID NO: 12 has been inserted on page 9. Support for this amendment can be found within the specification on page 19, lines 26 to 30 and in Claim 8 on page 46, lines 25 to 28.

Claim 6 has been amended recite that one or more amino acids of the tetrapeptide Arg-Asp-Ile-Thr are deleted. Support for this amendment can be found on page 7, lines 20 to 25, and on page 17, lines 23 to 27.

The paragraph beginning on page 36, line 2 has been amended to replace the designations Arg1653-Tyr1664 with Arg1652-Tyr1664. Support for this amendment may be found on page 36, lines 7 and 10.

Tables 1 and 2 have been amended to replace the commas serving as decimal points in some of the columns with periods to be consistent with the U.S. practice of using periods as decimal points. Table 1 was further amended to place a single SEQ ID in column (a) of the table. Support for this amendment can be found in the specification as filed on pages 17 to 24, where the "P" number used in the RAP designations in these tables are listed next to the corresponding SEQ ID NO.

A Sequence Listing has been added in accordance with 37 C.F.R. 1.821-1.825. Each of the sequences in the Sequence Listing appears in the application as originally filed.

No new matter has been added herewith. The changes made to the specification and claims by the current amendment, including **[deletions]** and additions, are shown on an attached sheet entitled **VERSION WITH MARKINGS TO SHOW CHANGES MADE**, which follows the signature page of this amendment.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410. A duplicate copy of this sheet is enclosed.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: Sept. 19, 2001

By: Daniel Hart

Daniel Hart
Registration No. 40,637
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620 Newport Center Drive
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification

The paragraph beginning on page 4, line 4, has been amended as follows:

Different animal models could be used as hemophilia dogs, SCID mice, hemophilia mice ... but until now, no satisfactory experimental model exists which makes it possible to forecast the immunogenicity or the immuno-modulatory effect of the FVIII preparations, or the susceptibility of the host, before they have been administered clinically.

The paragraph beginning on page 8, line 30, has been amended as follows:

possibly deleted from one or more amino acids from the heptapeptide **[Gly]**Glu-Thr-Lys-Ser-Trp-Phe-Thr or from the tripeptide Cys-Arg-Ala,

The following paragraph has been inserted on page 9, after line 23:

Glu Gly Ser Leu Ala Lys Glu Lys Thr Gln Thr Leu ,

The paragraph beginning on page 10, line 6, has been amended as follows:

Asp Ser Cys Pro Glu Glu Pro Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp [ ]Asp Leu Thr Asp Ser Glu Met ,

The paragraph beginning on page 10, line 18, has been amended as follows:

Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly Pro Gln Arg Ile Gly Arg Lys [ ]Tyr Lys Lys ,

The paragraph beginning on page 11, line 8, has been amended as follows:

Ser Cys Asp Lys Asn Thr Gly Asp Tyr **[Try]**Tyr Gly Asp Ser Tyr Glu Asp ,

The paragraph beginning on page 21, line 12, has been amended as follows:

Ser Cys Asp Lys Asn Thr Gly Asp Tyr **[Try]**Tyr Gly Asp Ser Tyr Glu Asp ,

The paragraph beginning on page 36, line 2 has been amended as follows:

In the clotting test, significant inhibition of FVIII activity was recorded in the presence of rabbit anti-(Cys^{329}-Asp^{348}) and anti-($Arg^{[1653]1652}$-Tyr^{1664}) antibodies, but different inhibition patterns were observed. Inhibition by anti-($Arg^{[1653]1652}$-Tyr^{1664}) follows second-order kinetics with a drastic reduction in FVIII activity. Anti-(Cys^{329}-Asp^{348}) Ig is less efficient and shows a more complex type of reaction, with a non-linear dependence on the antibody concentration. Epitope Arg^{1652}-Tyr^{1664} and the adjacent major binding site vWF (residues Glu^{1675}-Glu^{1684}) are located in the acidic light-chain peptide a3. As shown by western blotting, a3 is released from the A3 domain after thrombin treatment, preventing further binding of anti-(Arg^{1652}-Tyr^{1664}) Ig to activated FVIII. Similar results have been reported by Shima et al (1991), who described the FVIII sequence Asp^{1663}-Ser^{1669} as a binding site of rabbit polyclonal antibodies neutralizing FVIII activity. Epitope Cys^{329}-Asp^{348} overlapped the acidic Asp^{348}-Lys^{362} sequence (in a1) described as adjacent to the activated protein C (Arg^{336}) and thrombin (Arg^{372}) cleavage sites. It is the target of human hemophilic inhibitors. Anti-(Asp^{348}-Lys^{362}) antibodies may interfere with proteolysis or with the FX interaction site (Met^{337}-Arg^{372}) (Saenko et al., 1999 and Scandella et al., 2000).

Tables 1 and 2, located on pages 38 to 41, have been amended as indicated (entitled **VERSION WITH MARKINGS TO SHOW CHANGES MADE**) on the attached pages following the final page of the "marked up" Claims. "Clean" versions of Table 1 and 2 immediately follow the "marked up" version of the Tables.

In the Claims

6. **(Amended)** An isolated or purified polypeptide comprising an epitope of factor VIII, wherein said epitope is selected from the group consisting of:

(a) the epitope arginine 1648 to tyrosine 1664 inclusive, defined by the following sequence:

SEQ ID NO: 1:

Arg Asp Ile Thr Arg Thr Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr ,

(b) the epitope of (a) wherein one or more amino acids of the tetrapeptide Arg-Asp-Ile-Thr or one or two of the last amino acids of the peptide Asp-Tyr are deleted;

(c) the epitope aspartic acid 1681 to arginine 1696 inclusive, defined by the following sequence:

SEQ ID NO: 2:

Asp Glu Asp Glu Asn Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg ,

(d) the epitope of (c) wherein one or more amino acids of the epitope Asp-Glu-Asp-Glu are deleted;

(e) the epitope threonine 1739 to tyrosine 1748 inclusive, defined by the following sequence:

SEQ ID NO: 3:

Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr;

(f) the epitope asparagine 1777 to phenylalanine 1785 inclusive, defined by the following sequence:

SEQ ID NO: 4:

Asn Gln Ala Ser Arg Pro Tyr Ser Phe ;

(g) the epitope of (f) wherein one or two amino acids of the terminal dipeptide Ser-Phe or the tetrapeptide Pro-Tyr-Ser-Phe are deleted;

(h) the epitope glutamic acid 1794 to tyrosine 1815 inclusive, defined by the following sequence:

SEQ ID NO: 5:

Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg Lys Asn Phe Val Lys Pro

Asn Glu Thr Lys Thr Tyr ;

(i) the epitope of (h) wherein one or more amino acids from the first tripeptide Glu-Asp-Gln or the first nonapeptide Glu-Asp-Gln-Arg-Gln-Gly-Ala-Glu-Pro are deleted;

(j) the epitope methionine 1823 to aspartic acid 1831, defined by the following sequence:

SEQ ID NO: 6:

Met Ala Pro Thr Lys Asp Glu Phe Asp;

(k) the epitope glutamic acid 1885 to phenylalanine 1891 inclusive, defined by the following sequence:

SEQ ID NO: 7:

Glu Thr Lys Ser Trp Tyr Phe ;

(l) the epitope glutamic acid 1885 to alanine 1901 inclusive, defined by the following sequence:

SEQ ID NO: 8:

Glu Thr Lys Ser Trp Phe Thr Glu Asn Met Glu Arg Asn Cys Arg Ala ;

(m) the epitope of (l) wherein one or more amino acids from the heptapeptide **[Gly]**Glu-Thr-Lys-Ser-Trp-Phe-Thr or from the tripeptide Cys-Arg-Ala are deleted;

(n) the epitope aspartic acid 1909 to arginine 1917 inclusive, defined by the following sequence:

SEQ ID NO: 9:

Asp Pro Thr Phe Lys Glu Asn Tyr Arg;

(o) the epitope comprised between serine 2018 and histidine 2031 inclusive, defined by the following sequence:

SEQ ID NO: 10:

Ser Asn Lys Cys Gln Thr Pro Leu Gly Met Ala Ser Gly His; and

(p) any of the preceding epitopes (a) to (o) wherein at least one amino acid has been deleted.

10. **(Amended)** An isolated or purified polypeptide comprising an epitope of factor VIII, wherein said epitope is selected from the group consisting of:

(a) the epitope aspartic acid 403 to lysine 425 inclusive, defined by the following sequence:

SEQ ID NO: 15:

Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly Pro Gln Arg

Ile Gly Arg Lys Tyr Lys Lys;

(b) the epitope of (a) wherein one or more amino acids of the tetrapeptide Asp-Asp-Arg-Ser are deleted;

(c) the epitope valine 517 to arginine 527 inclusive, defined by the following sequence:

SEQ ID NO: 16:

Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg;

(d) the epitope of (c) wherein one or the two amino acids of the dipeptide Pro-Arg are deleted;

(e) the epitope tyrosine 555 to glutamine 565 inclusive defined by the following sequence:

SEQ ID NO: 17:

Tyr Lys Glu Ser Val Asp Gly Arg Gly Asn Gln;

(f) the epitope histidine 693 to glycine 701 inclusive, defined by the following sequence:

SEQ ID NO: 18

His Asn Ser Asp Phe Arg Asn Arg Gly;

(g) the epitope serine 710 to aspartic acid 725 inclusive, defined by the following sequence:

SEQ ID NO: 19

Ser Cys Asp Lys Asn Thr Gly Asp Tyr **[Try]**Tyr Gly Asp Ser Tyr Glu Asp;

(h) the epitope leucine 730 to serine 741 inclusive, defined by the following sequence:

SEQ ID NO: 20:

Leu Leu Ser Lys Asn Asn Ala Ile Glu Pro Arg Ser;

(i) the epitope of (h) wherein the terminal amino acid serine and/or the first amino acid leucine are deleted;

(j) the epitope serine 817 to serine 830 inclusive, defined by the following sequence:

SEQ ID NO: 21:

Ser Asp Asp Pro Ser Gly Ala Ile Asp Ser Asn Asn Ser; and

(k) any of the preceding epitopes (a) to (j) wherein at least one amino acid has been deleted.

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Table 1. Characterization of rabbit anti-FVIII peptides antibodies

SEQ ID(a)	Rabbit Antiserum(b)	ELISA Titer(c) [i.]		FVIII domain recognize(d)	RAP-IgG Recovery(e) µg/ml serum	Inhibitor Titer(f) BU/mg
		P-KLH	r-FVIII			
SEQ ID 11	RAP1	2.5	2.2	A1	27	-
SEQ ID 14	RAP2	3.6	2.5	A1/a1	55	1[,]±5
SEQ ID 15 [SEQ]	RAP3	2.5	3.2	A2	268	-
SEQ ID 19 [SEQ ID 21]	RAP4	2.5	1.3	A2/a2	12	-
SEQ ID 21[C]	RAP5	4.6	3.9	B	106	-
SEQ C [ID 01 SEQ]	RAP6	3.8	2.9	-	14	-
[ID 02]SEQ ID 01[5]	RAP7	3.9	3.9	a3 ↓	35	0[,]±5
SEQ ID 02[3 SEQ]	RAP8	1.9	0.9	a3/A3 ↓	3	-

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SEQ ID 05**[22]**	RAP9	3.8	2.6	A3	42	-
SEQ ID 23**[6 SEQ]**	RAP10	3.9	0.8	-	65	-
[ID 27]SEQ ID 22**[8]**	RAP11	ND	ND	ND	ND	ND
SEQ ID 26**[31 SEQ]**	RAP12	4.1	1[,].1	C2	ND	ND
[ID 32] SEQ ID 27 **[33]**	RAP13	3.7	1[,].1	C2	ND	ND
SEQ ID 28	RAP14	3.8	0[,].9	C2	ND	ND
SEQ ID 31	RAP15	3.2	0[,].7	C2	ND	ND
SEQ ID 32	RAP16	3.5	1[,].8	C2	ND	ND
SEQ ID 33	RAP17	4.8	1[,].2	C2	ND	ND

VERSION WITH MARKINGS TO SHOW CHANGES MADE

[2.]Table 2. Characterization of human anti-FVIII peptides autoantibodies

SEQ ID(a)	Human Anti-peptide Ig(b)	FVIII reactivity on immunoblot (-thrombin)(c)	(+thrombin)(d)	FVIII domain(e)	HAP-IgG Recovery(f) µg/10 mgIgG	FVIII inhibitory Activity(g) BU/mg
SEQ ID 11	HAP1	>92kDa	50kDa	A1	0[,]27	-
SEQ ID 14	HAP2	>92kDa	50kDa	A1/a1	1[,]07	3[,]4
SEQ ID 15	HAP3	>92kDa	44kDa	A2	0[,]06	-
SEQ ID 19	HAP4	92kDa	44kDa	A2/a2	0[,]12	+
SEQ ID 21	HAP5	>100kDa	-	B	0[,]26	-
SEQ C	HAP6	-	-	-	0[,]03	-
SEQ ID 01	HAP7	80kDa	80kDa	a3 ↓	0[,]20	-
SEQ ID 02	HAP8	80kDa	80kDa	a3/A3 ↓	0[,]01	+
SEQ ID 05	HAP9	80kDa	72kDa	A3	0[,]08	+
SEQ ID 23	HAP10	-	-	-	0[,]11	-
SEQ ID 22	HAP11	ND	ND	ND	0[,]98	4[,]3
SEQ ID 26	HAP12	ND	ND	ND	ND	ND

VERSION WITH MARKINGS TO SHOW CHANGES MADE

SEQ ID 27	HAP13	ND	ND	ND	ND	ND
SEQ ID 28	HAP14	ND	ND	ND	ND	ND
SEQ ID 31	HAP15	ND	ND	ND	ND	ND
SEQ ID 32	HAP16	80kDa	72 kDa	A3C1C2	2[,]±40	6[,]±3
SEQ ID 33	HAP17	ND	ND	ND	1[,]±06	2[,]±4

+: Inhibition >25% at 100μg/ml

Table 1. Characterization of rabbit anti-FVIII peptides antibodies

SEQ ID(a)	Rabbit Antiserum(b)	ELISA Titer(c)		FVIII domain recognize(d)	RAP-IgG Recovery(e) µg/ml serum	Inhibitor Titer(f) BU/mg
		P-KLH	r-FVIII			
SEQ ID 11	RAP1	2.5	2.2	A1	27	-
SEQ ID 14	RAP2	3.6	2.5	A1/a1	55	1.5
SEQ ID 15	RAP3	2.5	3.2	A2	268	-
SEQ ID 19	RAP4	2.5	1.3	A2/a2	12	-
SEQ ID 21	RAP5	4.6	3.9	B	106	-
SEQ C	RAP6	3.8	2.9	-	14	-
SEQ ID 01	RAP7	3.9	3.9	a3 ↓	35	0.5
SEQ ID 02	RAP8	1.9	0.9	a3/A3 ↓	3	-
SEQ ID 05	RAP9	3.8	2.6	A3	42	-
SEQ ID 23	RAP10	3.9	0.8	-	65	-
SEQ ID 22	RAP11	ND	ND	ND	ND	ND
SEQ ID 26	RAP12	4.1	1.1	C2	ND	ND

SEQ ID 27	RAP13	3.7	1.1	C2	ND	ND
SEQ ID 28	RAP14	3.8	0.9	C2	ND	ND
SEQ ID 31	RAP15	3.2	0.7	C2	ND	ND
SEQ ID 32	RAP16	3.5	1.8	C2	ND	ND
SEQ ID 33	RAP17	4.8	1.2	C2	ND	ND

Table 2. Characterization of human anti-FVIII peptides autoantibodies

SEQ ID(a)	Human Anti-peptide Ig(b)	FVIII reactivity on immunoblot (-thrombin)(c)	(+thrombin)(d)	FVIII domain(e)	HAP-IgG Recovery(f) µg/10 mgIgG	FVIII inhibitory Activity(g) BU/mg
SEQ ID 11	HAP1	>92kDa	50kDa	A1	0.27	-
SEQ ID 14	HAP2	>92kDa	50kDa	A1/a1	1.07	3.4
SEQ ID 15	HAP3	>92kDa	44kDa	A2	0.06	-
SEQ ID 19	HAP4	92kDa	44kDa	A2/a2	0.12	+
SEQ ID 21	HAP5	>100kDa	-	B	0.26	-
SEQ C	HAP6	-	-	-	0.03	-
SEQ ID 01	HAP7	80kDa	80kDa	a3 ↓	0.20	-
SEQ ID 02	HAP8	80kDa	80kDa	a3/A3 ↓	0.01	+
SEQ ID 05	HAP9	80kDa	72kDa	A3	0.08	+
SEQ ID 23	HAP10	-	-	-	0.11	-
SEQ ID 22	HAP11	ND	ND	ND	0.98	4.3
SEQ ID 26	HAP12	ND	ND	ND	ND	ND

SEQ ID 27	HAP13	ND	ND	ND	ND	ND
SEQ ID 28	HAP14	ND	ND	ND	ND	ND
SEQ ID 31	HAP15	ND	ND	ND	ND	ND
SEQ ID 32	HAP16	80kDa	72 kDa	A3C1C2	2.40	6,3
SEQ ID 33	HAP17	ND	ND	ND	1.06	2.4

+: Inhibition >25% at 100μg/ml